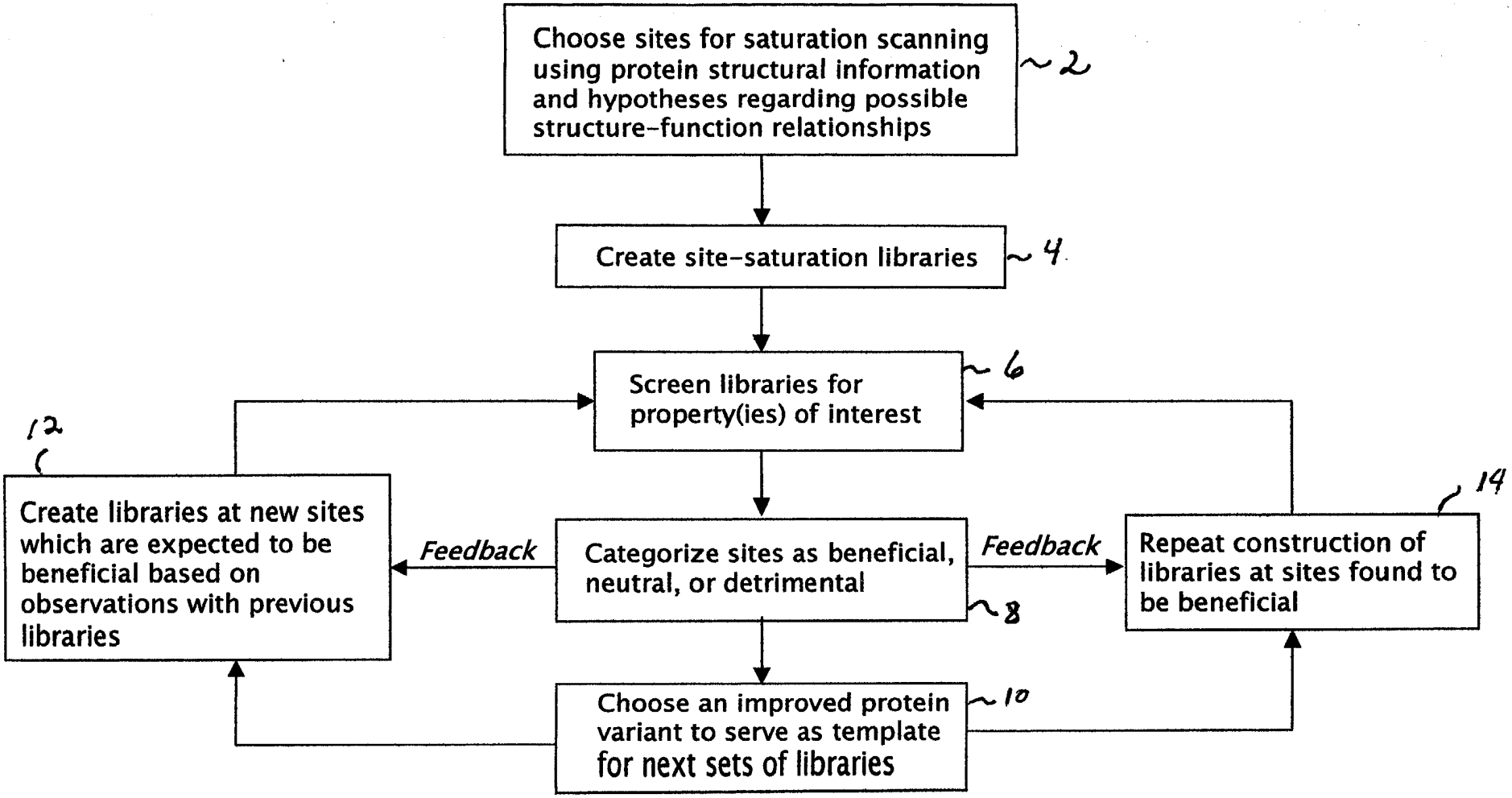

Choose sites for saturation scanning using protein structural information and hypotheses regarding possible structure-function relationships
2
Create site-saturation libraries
4
Screen libraries for property(ies) of interest
6
12
Create libraries at new sites which are expected to be beneficial based on observations with previous libraries
Feedback
Categorize sites as beneficial, neutral, or detrimental
8
Feedback
14
Repeat construction of libraries at sites found to be beneficial
Choose an improved protein variant to serve as template for next sets of libraries
10

FIG. 1

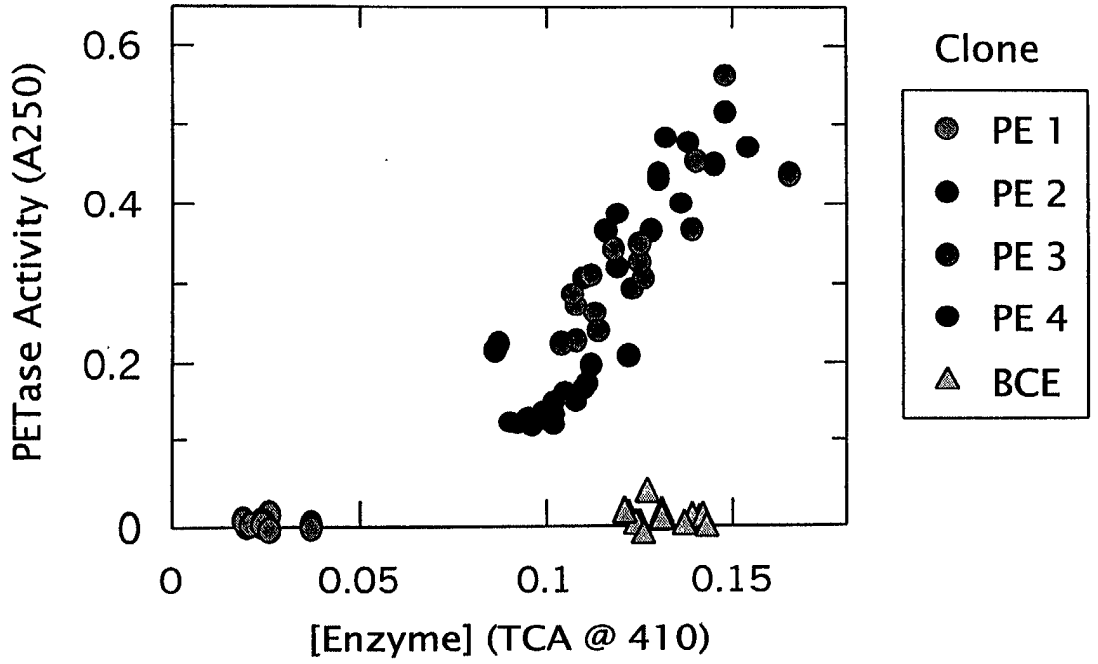


FIG. 2

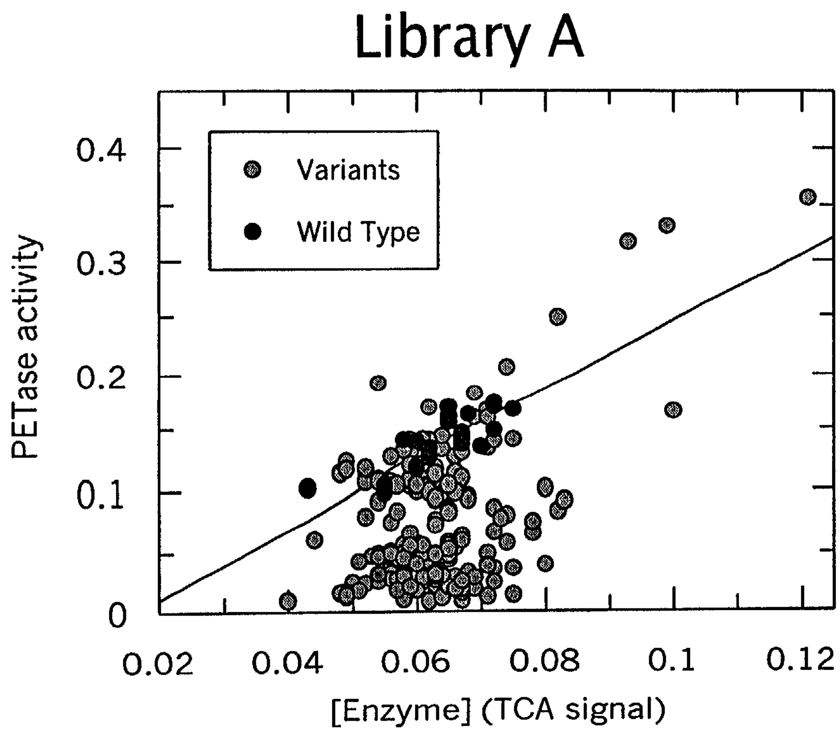
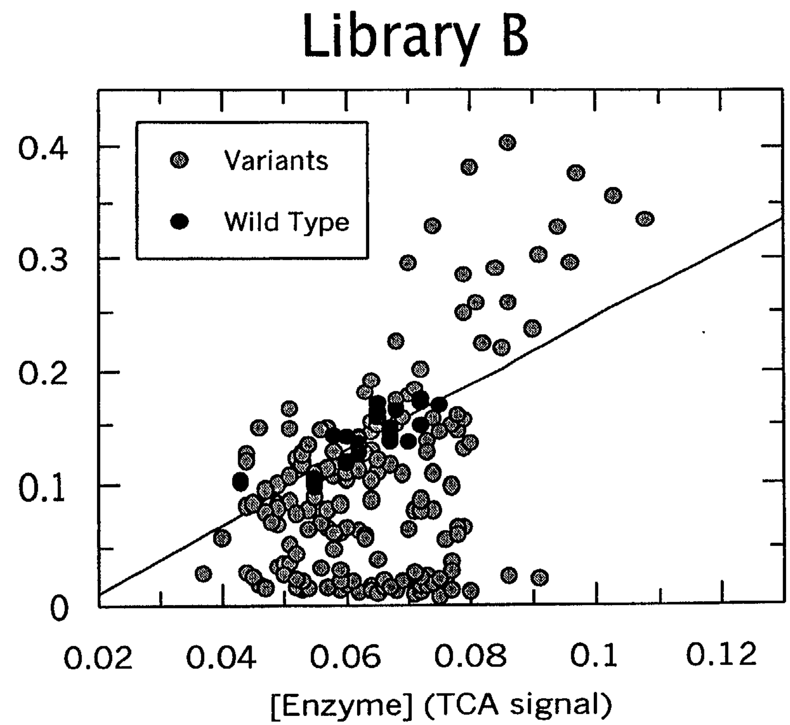

Library A
Variants
Wild Type
PETase activity
0.4
0.3
0.2
0.1
0
0.02
0.04
0.06
0.08
0.1
0.12
[Enzyme] (TCA signal)

FIG. 3


Library B
Variants
Wild Type
0.4
0.3
0.2
0.1
0
0.02
0.04
0.06
0.08
0.1
0.12
[Enzyme] (TCA signal)

] **FIG. 4**

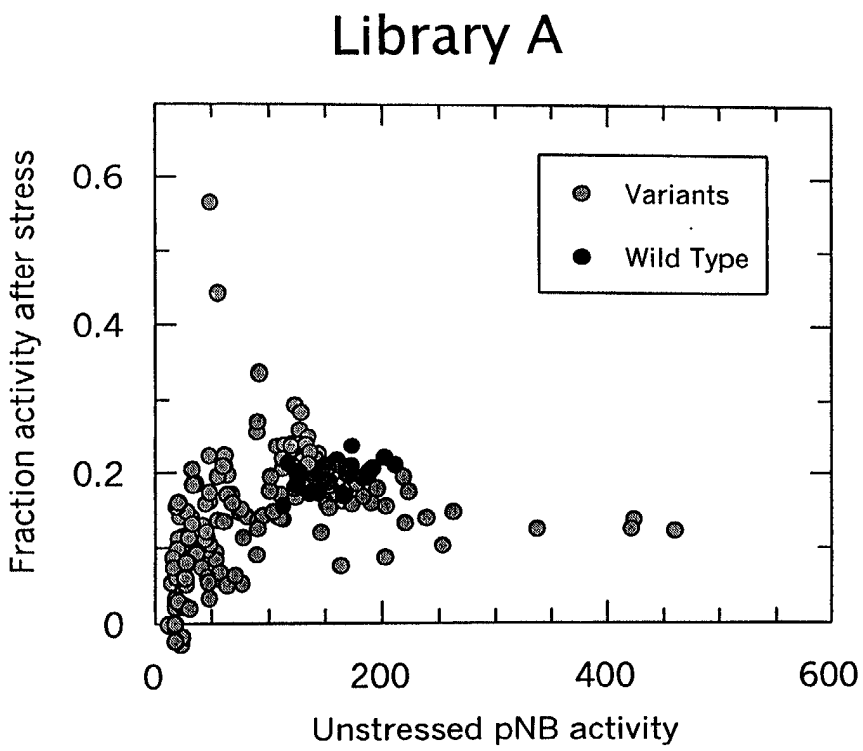


FIG. 5

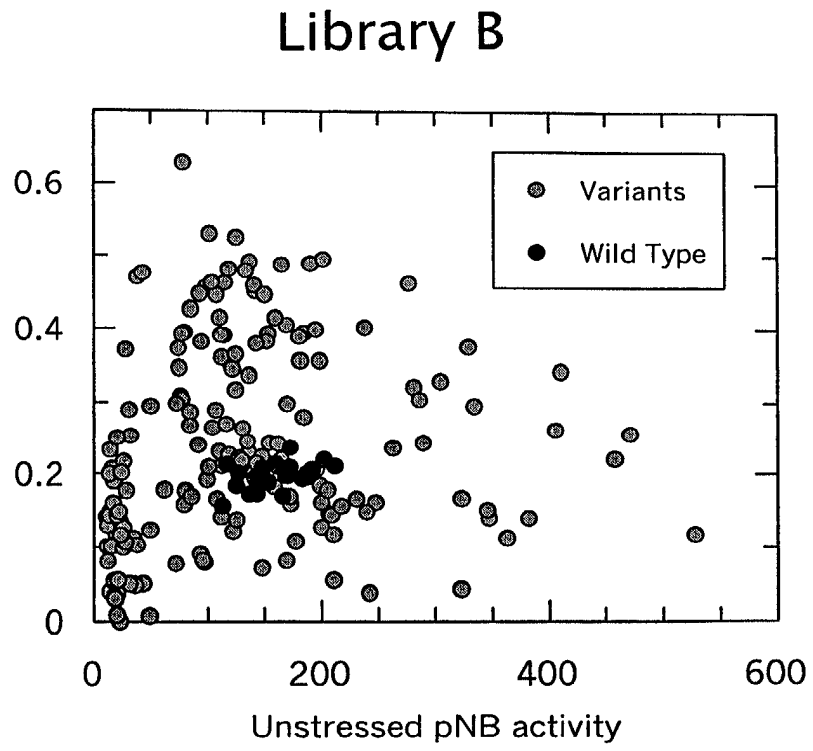


FIG. 6

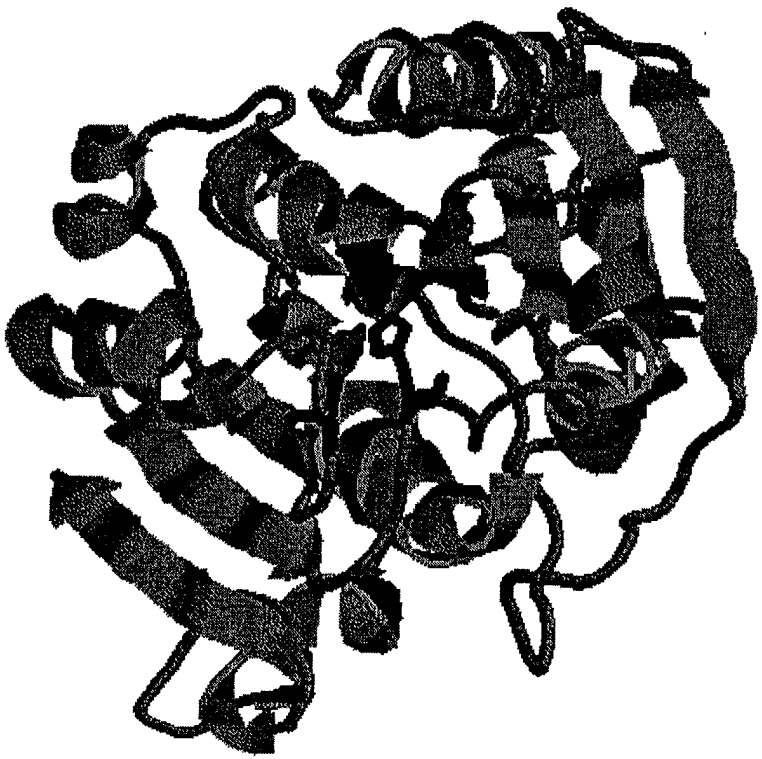


FIG. 7

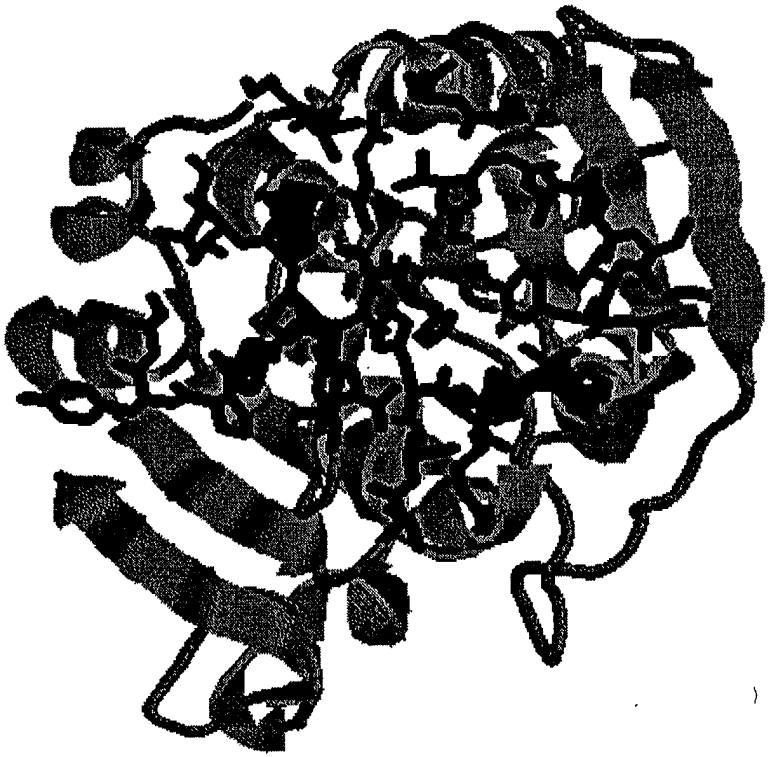


FIG. 8

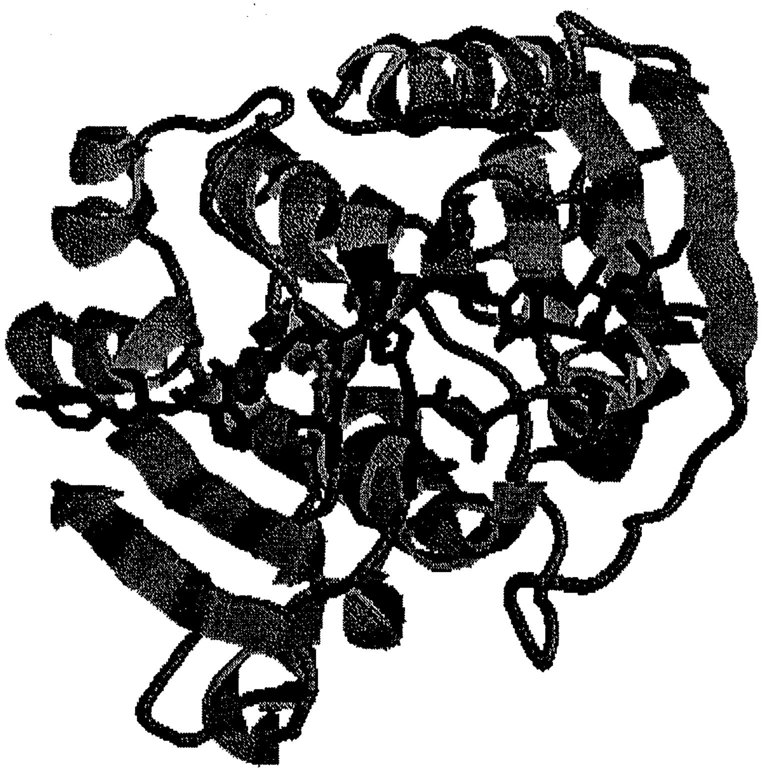


FIG. 9

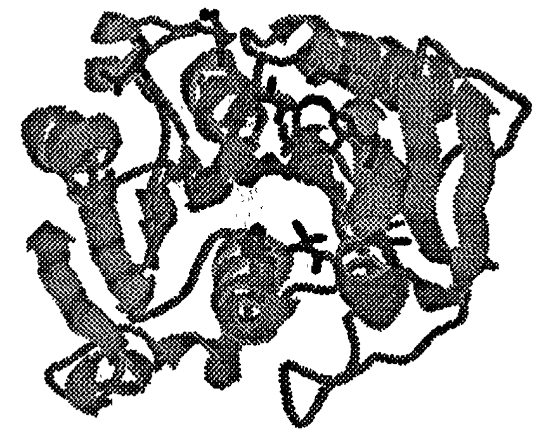


FIG. 10

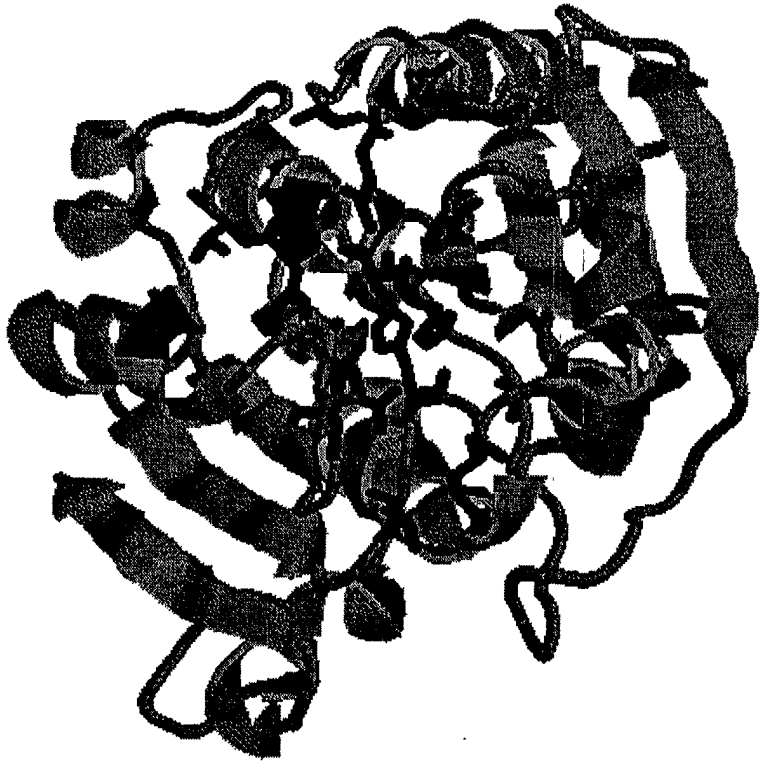


FIG. 11

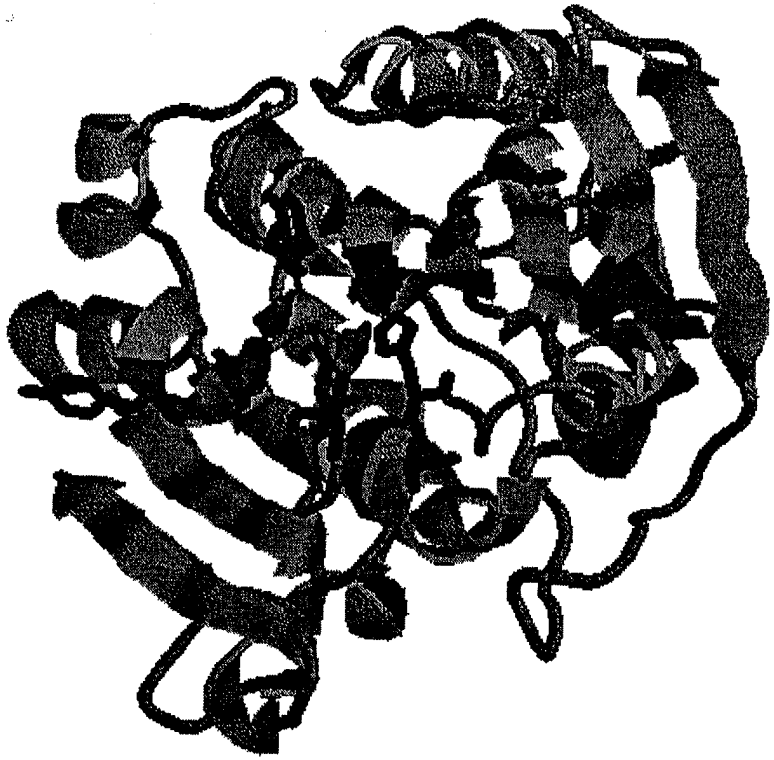


FIG. 12

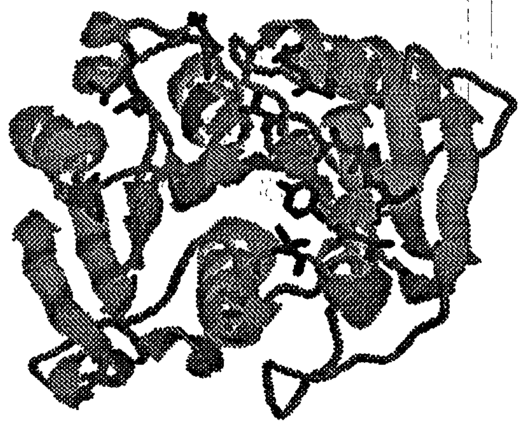


FIG. 13

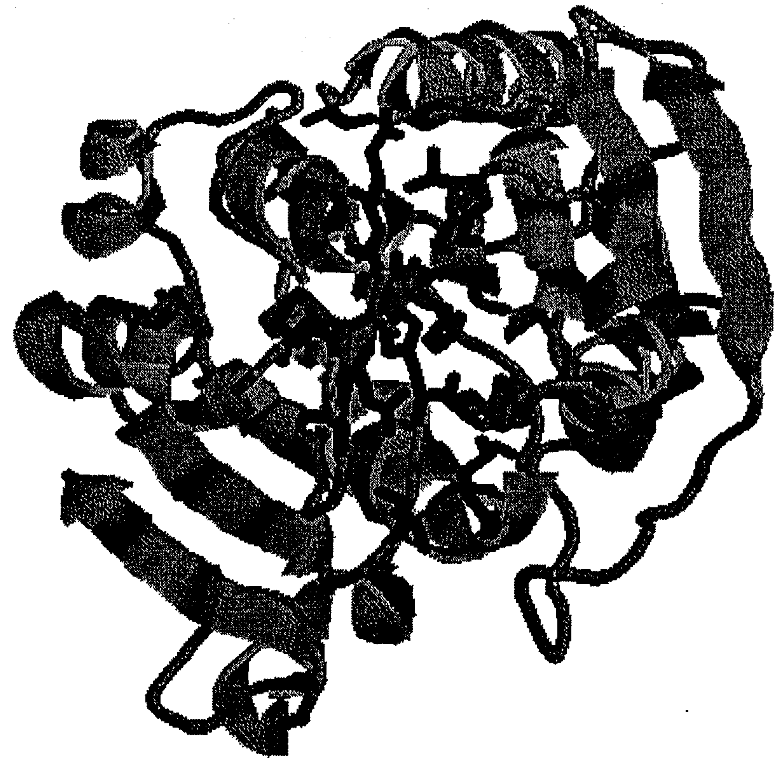


FIG. 14

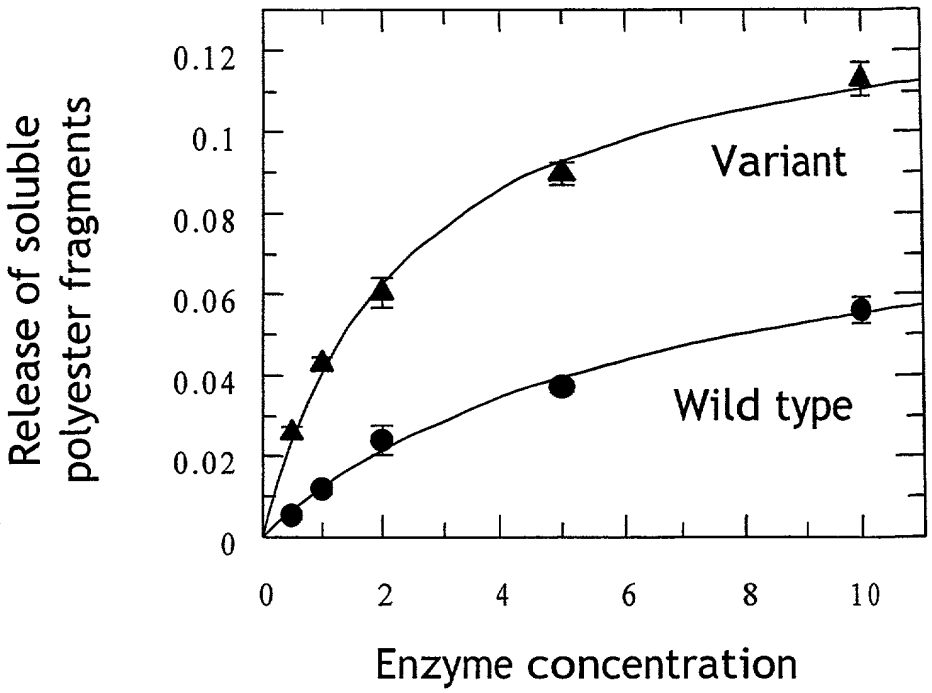

Release of soluble polyester fragments
0.12
0.1
0.08
0.06
0.04
0.02
0
Variant
Wild type
0
2
4
6
8
10
Enzyme concentration

FIG. 15

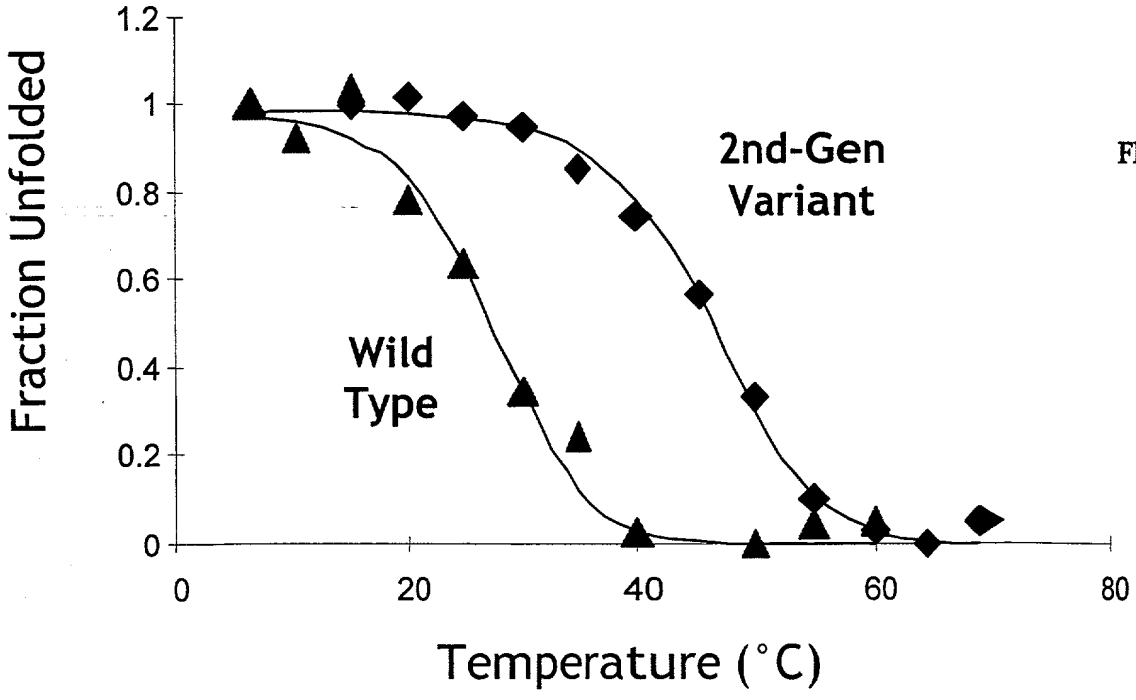

Fraction Unfolded
1.2
1
0.8
0.6
0.4
0.2
0
2nd-Gen Variant
Wild Type
0
20
40
60
80
Temperature (°C)

FIG. 16

Created: Tuesday, February 01, 2000 2:19 PM

```
        10         20         30         40
TGGCGGCCTCTTGCCTGTCCGTCTGTGCCACTGTCGCGGC 40
GGCTCCCCTGCCGGATACACCGGGAGCGCCATTTCCGGCT 80
GTCGCCAATTTCGACCGCAGTGGCCCCTACACCACCAGCA 120
GCCAGAGCGAGGGGCCGAGCTGTCGCATCTATCGGCCCCG 160
CGACCTGGGTCAGGGGGGCGTGCGTCATCCGGTGATTCTC 200
        210        220        230        240
TGGGGCAATGGCACCGGTGCCGGGCCGTCCACCTATGCCG 240
GCTTGCTATCGCACTGGGCAAGCCACGGTTTCGTGGTGGC 280
GGCGGCGGAAACCTCCAATGCCGGTACCGGGCGGGAAATG 320
CTCGCCTGCCTGGACTATCTGGTACGTGAGAACGACACCC 360
CCTACGGCACCTATTCCGGCAAGCTCAATACCGGGCGAGT 400
        410        420        430        440
CGGCACTTCTGGGCATTCCCAGGGTGGTGGCGGCTCGATC 440
ATGGCCGGGCAGGATACGAGGGTGCGTACCACGGCGCCGA 480
TCCAGCCCTACACCCTCGGCCTGGGGCACGACAGCGCCTC 520
GCAGCGGCGGCAGCAGGGGCCGATGTTCCTGATGTCCGGT 560
GGCGGTGACACCATCGCCTTTCCCTACCTCAACGCTCAGC 600
        610        620        630        640
CGGTCTACCGGCGTGCCAATGTGCCGGTGTTCTGGGGCGA 640
ACGGCGTTACGTCAGCCACTTCGAGCCGGTCGGTAGCGGT 680
GGGGCCTATCGCGGCCCGAGCACGGCATGGTTCCGCTTCC 720
AGCTGATGGATGACCAAGACGCCCGCGCTACCTTCTACGG 760
CGCGCAGTGCAGTCTGTGCACCAGCCTGCTGTGGTCGGTC 800
        810        820        830        840
GAGCGCCGCGGGCTTTAA 818
```

Fig. 17

lipasewtgene Map.MPD (1 > 818) Site and Sequence
Enzymes : All 515 enzymes (No Filter)
Settings : Circular, Certain Sites Only, Standard Genetic Code

```
TGGCGGCCTCTTGCCTGTCCGTCTGTGCCACTGTCGCGGCGGCTCCCCTGCCGGATACACCGG 63
Met Ala Ala Ser Cys Leu Ser Val Cys Ala Thr Val Ala Ala Ala Pro Leu Pro Asp Thr Pro

GAGCGCCATTTCCGGCTGTCGCCAATTTCGACCGCAGTGGCCCCTACACCACCAGCAGCCAGA 126
Gly Ala Pro Phe Pro Ala Val Ala Asn Phe Asp Arg Ser Gly Pro Tyr Thr Thr Ser Ser Gln

GCGAGGGGCCGAGCTGTCGCATCTATCGGCCCCGCGACCTGGGTCAGGGGGGCGTGCGTCATC 189
Ser Glu Gly Pro Ser Cys Arg Ile Tyr Arg Pro Arg Asp Leu Gly Gln Gly Gly Val Arg His

CGGTGATTCTCTGGGGCAATGGCACCGGTGCCGGGCCGTCCACCTATGCCGGCTTGCTATCGC 252
Pro Val Ile Leu Trp Gly Asn Gly Thr Gly Ala Gly Pro Ser Thr Tyr Ala Gly Leu Leu Ser

ACTGGGCAAGCCACGGTTTCGTGGTGGCGGCGGCGGAAACCTCCAATGCCGGTACCGGGCGGG 315
His Trp Ala Ser His Gly Phe Val Val Ala Ala Ala Glu Thr Ser Asn Ala Gly Thr Gly Arg

AAATGCTCGCCTGCCTGGACTATCTGGTACGTGAGAACGACACCCCCTACGGCACCTATTCCG 378
Glu Met Leu Ala Cys Leu Asp Tyr Leu Val Arg Glu Asn Asp Thr Pro Tyr Gly Thr Tyr Ser

GCAAGCTCAATACCGGGCGAGTCGGCACTTCTGGGCATTCCCAGGGTGGTGGCGGCTCGATCA 441
Gly Lys Leu Asn Thr Gly Arg Val Gly Thr Ser Gly His Ser Gln Gly Gly Gly Gly Ser Ile

TGGCCGGGCAGGATACGAGGGTGCGTACCACGGCGCCGATCCAGCCCTACACCCTCGGCCTGG 504
Met Ala Gly Gln Asp Thr Arg Val Arg Thr Thr Ala Pro Ile Gln Pro Tyr Thr Leu Gly Leu

GGCACGACAGCGCCTCGCAGCGGCGGCAGCAGGGGCCGATGTTCCTGATGTCCGGTGGCGGTG 567
Gly His Asp Ser Ala Ser Gln Arg Arg Gln Gln Gly Pro Met Phe Leu Met Ser Gly Gly Gly

ACACCATCGCCTTTCCCTACCTCAACGCTCAGCCGGTCTACCGGCGTGCCAATGTGCCGGTGT 630
Asp Thr Ile Ala Phe Pro Tyr Leu Asn Ala Gln Pro Val Tyr Arg Arg Ala Asn Val Pro Val

TCTGGGGCGAACGGCGTTACGTCAGCCACTTCGAGCCGGTCGGTAGCGGTGGGGCCTATCGCG 693
Phe Trp Gly Glu Arg Arg Tyr Val Ser His Phe Glu Pro Val Gly Ser Gly Gly Ala Tyr Arg

GCCCGAGCACGGCATGGTTCCGCTTCCAGCTGATGGATGACCAAGACGCCCGCGCTACCTTCT 756
Gly Pro Ser Thr Ala Trp Phe Arg Phe Gln Leu Met Asp Asp Gln Asp Ala Arg Ala Thr Phe

ACGGCGCGCAGTGCAGTCTGTGCACCAGCCTGCTGTGGTCGGTCGAGCGCCGCGGGCTTTAA 818
Tyr Gly Ala Gln Cys Ser Leu Cys Thr Ser Leu Leu Trp Ser Val Glu Arg Arg Gly Leu •
```

Fig 18